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COMPARATIVE TOXICITY OF FLUORANTHENE AND PENTACHLOROBENZENE TO THREE FRESHWATER INVERTEBRATES

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Abstract—This study examined the temporal component of pentachlorobenzene lethal body residues among three freshwater invertebrates. Also, using previous fluoranthene data allowed a more detailed examination of the role of biotransformation in lethal body residues and comparisons of lethal residues across chemical classes. Time-dependent toxicity of fluoranthene and pentachlorobenzene were compared among *Hyalella azteca*, *Chironomus tentans*, and *Diporeia* spp. Lethal body residues required for 50% mortality (LR50) were not constant and decreased with exposure time for all species. Fluoranthene was most toxic to *C. tentans* with LR50 values of 0.38 $\mu mol \cdot g^{-1}$ at 2 d to 0.15 $\mu mol \cdot g^{-1}$ at 10 d and least toxic to *Diporeia* spp. with values of 9.97 $\mu mol \cdot g^{-1}$ at 10 d to 3.67 $\mu mol \cdot g^{-1}$ at 28 d. The LR50 values for *H. azteca* were intermediate and ranged from 2.25 $\mu mol \cdot g^{-1}$ at 5 d to 0.56 $\mu mol \cdot g^{-1}$ at 28 d. Pentachlorobenzene LR50 values were less variable among species and ranged from 1.20 $\mu mol \cdot g^{-1}$ at 4 d to 0.81 $\mu mol \cdot g^{-1}$ at 10 d for *C. tentans*, 5.0 $\mu mol \cdot g^{-1}$ at 20 d and 2.75 $\mu mol \cdot g^{-1}$ at 28 d for *Diporeia* spp., and 1.51 $\mu mol \cdot g^{-1}$ at 4 d and 0.71 $\mu mol \cdot g^{-1}$ at 28 d for *H. azteca*. When LR50 values for fluoranthene and pentachlorobenzene were compared at steady state, the lethal residues for the amphipod species were within the range expected for nonpolar narcotic chemicals (anesthetics); however, *C. tentans* was more sensitive to fluoranthene than pentachlorobenzene, confirming our previous hypothesis that biotransformation of fluoranthene likely produces a metabolite(s) acting by some specific mechanism of action. The information collected from this study allows a greater understanding of residue–response relationships, specifically relative species sensitivities.

Keywords—Aquatic invertebrates Fluoranthene Pentachlorobenzene Lethal body residue Time-dependent toxicity

INTRODUCTION

The lethal body residue has been proposed as an alternative dose metric for evaluating the toxicity of chemicals to aquatic organisms over the more traditional concentration-based toxicity measurements. The advantages of using the molar contaminant concentrations in the tissues of the organism over the traditional approach are the implicit consideration of both contaminant bioavailability and multiple exposure routes [1]. For short-term exposures, the toxicity of nonpolar narcotic chemicals acting via anesthesia based on internal residue concentrations has been shown to be relatively constant for acute lethal narcosis in fish ranging from 2 to 8 $mmol \cdot kg^{-1}$ [1,2]. Despite the apparent threshold suggested from these acute mortality studies, recent work has demonstrated that for compounds with a narcotic mechanism of action, the body residue responsible for 50% mortality varies with exposure time for fish [3–5], crabs [6], and amphipods [7–9]. Several explanations have been proposed to account for this temporal variation in the lethal body residue, including shifts in mechanism of action [10]; increased stress [4]; partitioning of contaminants within tissues, particularly the storage lipids; and potential influence of biotransformation processes [9].

Recent examinations of the time-dependent toxicity of narcotic chemicals have suggested that the noted decrease in lethal body residue with time can be explained as a result of a combination of toxicokinetic and toxicodynamic processes [8,9,11]. In these studies, the toxicity is thought to occur as a result of a buildup of damage that cannot be explained by toxicokinetic processes alone. These temporal changes were modeled using the damage assessment model (DAM) to account for both the influence of the toxicokinetics and, more important, the influence of the toxicodynamics [11]. Assuming first-order kinetics and a narcotic mode of action, the model clearly demonstrates the importance of the rate of damage formation early in the exposure since the damage repair would be considered negligible for short exposure times. In longer exposures, the model shows the importance of the repair process, represented by the rate constant for damage repair (k_r). It follows that it is the damage producing mortality that is constant, not the body residue, as has been proposed for nonpolar narcosis [12–14]. Therefore, defining the temporal dependence of lethal body residues should improve the utility and application of the body residue as a dose metric for adverse effects in aquatic biota.

In a previous study examining the toxicity of fluoranthene to *Hyalella azteca* and *Chironomus tentans*, the acute lethal residues differed by approximately an order of magnitude. Although both species biotransformed fluoranthene fairly rapidly, the lethal residues for *H. azteca* expressed as fluoranthene total equivalents (parent compound + metabolites) were in the range expected for compounds acting via narcosis, suggesting that the biotransformation products were acting by narcosis [9]. However, for *C. tentans*, the body residues required to produce mortality were more in line with lethal residues from compounds that act by a specific mechanism of action (e.g.,

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acetylcholinesterase inhibitors, respiratory inhibitors, neurotoxins, and so on) [1]. This seems to indicate that some of the biotransformation products may have contributed to the increased toxicity reflected by a shift in the mechanism of toxic action. However, this assumption of a mode of action other than narcosis for fluoranthene in *C. tentans* could not be validated because body residue data available for comparison are lacking.

The main objective of this study was to evaluate the temporal component of lethal body residues for pentachlorobenzene to three freshwater invertebrate species at differing exposure intervals up to 28 d. In addition, the pentachlorobenzene data will be compared to previous body residue data to examine the potential impact of biotransformation on the body residue response relationship and in particular to demonstrate whether *C. tentans* is simply more sensitive to narcotic contaminants or to confirm that a biotransformation product likely contributes to the response by this species.

MATERIALS AND METHODS

Organisms

Three invertebrate species were selected: the amphipods *H. azteca* (juvenile) and *Diporeia* spp. (juvenile) and the midge *C. tentans* (third instar). *Hyalella azteca* and *C. tentans* were chosen because they have been recommended by the U.S. Environmental Protection Agency (U.S. EPA) for sediment toxicity testing [15] and because of their ecological importance and geographical distribution. The amphipod *Diporeia* spp. was selected because the organism is an important component of the Great Lakes (USA) food webs and its biology differs from that of the other two species in that *Diporeia* has minimal ability to biotransform polycyclic aromatic hydrocarbons [9,16]. *Hyalella azteca* and *C. tentans* were cultured at Southern Illinois University–Carbondale (Carbondale, IL, USA) in accordance with standardized methods [15]. Two- to three-week old *H. azteca* were harvested for use by sieving mixed-age organisms. Organisms were considered the correct size if they passed through the 1,000-μm sieve but were retained on a 500-μm sieve. Third-instar *C. tentans* were obtained from collected egg masses. *Diporeia* spp. were field collected using a Ponar grab from an 80-m-deep station on Lake Michigan (43°11.747′N, 86°28.957′W) that has been shown to have low sediment contaminant concentrations [17]. The field-collected *Diporeia* were acclimated to 7°C under darkness for one week to approximate their natural conditions and eliminate organisms possibly injured during collection and transport (see Schuler et al. [9] for detailed methodology on handling of test organisms).

Chemicals

The ^{14}C-radiolabeled pentachlorobenzene and fluoranthene (specific activity 10 mCi·mmol^{-1} and 44 mCi·mmol^{-1}, respectively) and ^{12}C-nonradiolabeled compounds were obtained from Sigma-Aldrich (St. Louis, MO, USA). The radiopurity of the two compounds were determined using a combination of high-pressure liquid chromatography (Agilent Model 1100 Atlanta, GA, USA) followed by liquid scintillation counting (LSC) using a Packard 1900TR liquid scintillation analyzer (Packard Instrument, Downers Grove, IL, USA). The high-pressure liquid chromatography separations were performed with a Zorbax SB-C18 column (5 μm, 4.6 × 150 mm), and separate fractions were collected using an Agilent Model 1100 fraction collector. Samples were counted on the LSC using automatic quench control, and sample counts were corrected for background and quench using the external standards ratio method. The pentachlorobenzene isotope purity was determined to be >96% and the fluoranthene purity >95%. Stock solutions were prepared by adding known quantities of radiolabeled compound to known amounts of nonradiolabeled compound using acetone as a carrier. Concentrations were determined on the basis of the specific activity recalculated by adjusting for the isotopic dilution.

Experimental design

Experiments were conducted as previously described [9]. Briefly, all experiments were conducted as water-only exposures. Moderately hard exposure water was prepared by adding the necessary salts to deionized water and then allowing the water to mix overnight to ensure the required water quality. *Diporeia* spp. exposures used 0.45-μm-filtered Huron River (Ann Arbor, MI, USA) water, which has characteristics similar to Lake Michigan water and has been previously used in bioassays [18]. The volume of the acetone carrier was the same for each exposure concentration and was less than 100 μl·L^{-1} in all exposures. Solvent controls received the same amount of acetone as the treatments.

The time-dependent acute toxicity for each species was examined using 10-d (*C. tentans*) and 28-d (*H. azteca* and *Diporeia*) exposures. Experiments were conducted in a Precision Scientific Environmental Chamber (Chicago, IL, USA) maintained at 23°C with a 16:8-h light:dark photoperiod (*H. azteca* and *C. tentans*), and *Diporeia* were exposed at 7°C with no light to approximate their natural habitat. All organisms were exposed to a series of five contaminant concentrations and a solvent control, predetermined from range-finding experiments. *Hyalella azteca* and *Diporeia* exposures were conducted using 250-ml beakers containing 200 ml of water. A total of 15 replicates and 10 organisms per replicate were used in each experiment, with five beakers remaining at 28 d. *Chironomus tentans* were exposed individually in 25-ml beakers containing a substrate of 1.5 g quartz sand (Fisher Scientific, Hampton, NH, USA) using 75 replicates per concentration at the beginning of each experiment with 50 beakers remaining at the end of the experiment. *Chironomus tentans* were exposed individually because of their cannibalistic nature and burrowing activities, which makes assessing daily mortality difficult.

Exposure water concentrations were monitored before and after each daily water change by removing a 2-ml water sample and counting via LSC. *Hyalella azteca* and *C. tentans* were fed 100 μl of a 40-g·L^{-1} ground TetraFin® (Tetra Holding, Blacksburg, VA, USA) daily. Mortality was assessed daily, and body residues were measured using LSC.

The accumulation kinetics were determined by randomly removing two replicates from each concentration at exposure times of 0, 0.5, 1, 2, 4, and 10 d for *H. azteca* and 0, 0.5, 1, 2, 4, and 10 d for *C. tentans*. Accumulation was determined for *Diporeia* spp. at 0, 1, 2, 4, 10, and 28 d. The organisms were then analyzed by placing them directly into scintillation cocktail, sonicating (Tekmar High-Intensity Ultrasonic Processor, Tekmar, Solon, OH, USA), and then counting using LSC. Sample blanks were included in each analysis to track any potential background contamination.

In a preliminary exposure, the biotransformation of pentachlorobenzene was assessed for the three test species fol-

Table 1. Estimated toxicokinetic parameters for pentachlorobenzene in *Hyalella azteca, Chironomus tentans,* and *Diporeia* spp.

Organism	Exposure concn. ($\mu g \cdot L^{-1}$)	k_u[a] ($ml \cdot g^{-1} \cdot h^{-1}$)	SD[b]	k_e[c] (h^{-1})	SD	Log BCF[d]
H. azteca	100	31.4	17.0	0.026	0.035	3.08
	200	26.0	7.56	0.019	0.014	3.14
	400	33.8	4.60	0.027	0.009	3.10
	600	40.9	5.06	0.040	0.007	3.01
	800	40.8	3.65	0.039	0.004	3.02
C. tentans	100	158.6	34.2	0.275	0.085	2.76
	200	184.0	45.9	0.221	0.068	2.92
	400	145.8	32.4	0.292	0.057	2.70
	600	162.7	43.9	0.353	0.065	2.66
	800	148.5	48.6	0.324	0.100	2.66
Diporeia spp.	100	21.7	10.5	0.004	0.003	3.73
	200	10.5	2.2	0.003	0.001	3.54
	400	10.1	2.6	0.004	0.002	3.40
	600	5.2	3.6	0.003	0.001	3.24
	800	77.2	9.6	0.030	0.009	3.41

[a] k_u = the uptake clearance coefficient ($ml \cdot g^{-1} \cdot h^{-1}$).
[b] SD = standard deviation.
[c] k_e = total compound elimination rate constant (h^{-1}).
[d] Bioconcentration factor (BCF) based on wet weight calculated from kinetic estimates using BCF = $k_u \cdot k_e^{-1}$ and has units of $ml \cdot g^{-1}$ wet weight.

lowing a 10-d exposure. Organisms were ground using a tissue homogenizer in ice-cold acetone, and the extracts were analyzed using HPLC methods as described previously for determining compound purity.

To maintain consistency with a previous study, fluoranthene biotransformation was examined using a modified lipid extraction technique, which allows for the determination of fluoranthene residues based on their affinity for the organic, aqueous, or bound fraction [9]. The organic fraction is expected to contain the toxic components (parent compound plus phase 1 metabolites), while the aqueous fraction should contain the nontoxic water-soluble phase 2 metabolites. Fluoranthene biotransformation was quantified with exposure time to verify the assumption that the parent fraction is constant with time. These data were used to calculate the toxic fraction of fluoranthene residues by correcting the total equivalent concentrations determined by LSC by the toxic fraction (parent and phase 1 metabolites) determined from the accumulation.

Total lipids for each species were measured using the colorimetric method described in Van Handel [19]. This allows for lipid normalization of the body residue data to determine its potential to reduce the variability of body residues within and among species.

Data analysis

Toxicokinetic parameters were determined using the time-weighted average of the pentachlorobenzene concentration in the water by fitting accumulation data to the following first-order two-compartment model (Eqn. 1) using a fourth-order Runga–Kutta approach in the software package Scientist, version 2.01 (MicroMath, St. Louis, MO, USA):

$$\frac{dC_a}{dt} = k_u C_w - k_e C_a \quad (1)$$

where C_a = concentration of total compound in the animal ($\mu mol \cdot g^{-1}$), C_w = time-weighted average of the chemical concentration in water ($\mu mol \cdot ml^{-1}$), k_u = uptake clearance coefficient ($ml \cdot g^{-1} \cdot h^{-1}$), k_e = total compound elimination rate constant (h^{-1}), and t = time (h).

Mortality data were analyzed by probit analysis or trimmed Spearman–Karber using SAS® (Cary, NC, USA) to the lethal body residue values ($LR50_{(t)}$) corresponding to 50% mortality based on residues determined from dead organisms.

The time dependence of the lethal residue data was examined by fitting the data to the following DAM proposed by Lee et al. [11]:

$$LR50_{(t)} = \frac{D_L/k_a}{\frac{1}{(1 - e^{-k_r \cdot t})} \times \left(\frac{e^{-k_r \cdot t} - e^{-k_e \cdot t}}{k_r - k_e} + \frac{1 - e^{-k_r \cdot t}}{k_r} \right)} \quad (2)$$

where $LR50_{(t)}$ = the time-dependent lethal residue ($\mu mol \cdot g^{-1}$), D_L/k_a = the toxic damage level required to cause 50% mortality ($\mu mol \cdot g^{-1} \cdot h^{-1}$), and k_r = the first-order rate constant for damage recovery (h^{-1}).

A portion of this paper will compare lethal body residues of pentachlorobenzene and fluoranthene in *C. tentans*, *H. azteca*, and *Diporeia*. Some of the lethal residue data for fluoranthene used in these comparisons were obtained from a previous study [9].

RESULTS

Toxicokinetics

The toxicokinetic values for uptake clearance rate, elimination rate, and bioconcentration factor (BCF) for pentachlorobenzene were measured for each of the three organisms at each exposure concentration (Table 1). The toxicokinetic parameters varied among species, with *C. tentans* having the fastest uptake clearance rates (k_u), ranging from 145.8 to 184.0 $ml \cdot g^{-1} \cdot h^{-1}$, followed by *H. azteca*, with values ranging from 26.0 to 40.9 $ml \cdot g^{-1} \cdot h^{-1}$. The values for *Diporeia* were more variable with k_u values ranging from 5.2 to 77.2 $ml \cdot g^{-1} \cdot h^{-1}$. Elimination rates for *Diporeia* were similarly variable, differing by approximately an order of magnitude (0.003–0.030 h^{-1}). The elimination rates of pentachlorobenzene were higher for *C. tentans* and *H. azteca* compared to *Diporeia* and ranged from 0.221 to $0.353 \cdot h^{-1}$ and 0.019 to $0.040 \cdot h^{-1}$, respectively.

Even considering some of the variability in the toxicoki-

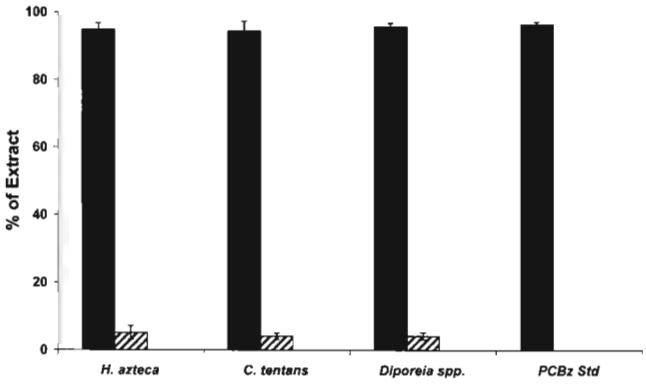

Fig. 1. Percent parent compound for *Hyalella azteca*, *Chironomus tentans*, and *Diporeia* following a 10-d water-only exposure to pentachlorobenzene (PCBz). TCBz = tetrachlorobenzene. PCBz; TCBz; other. Black = PCB7; hatched = TCB7.

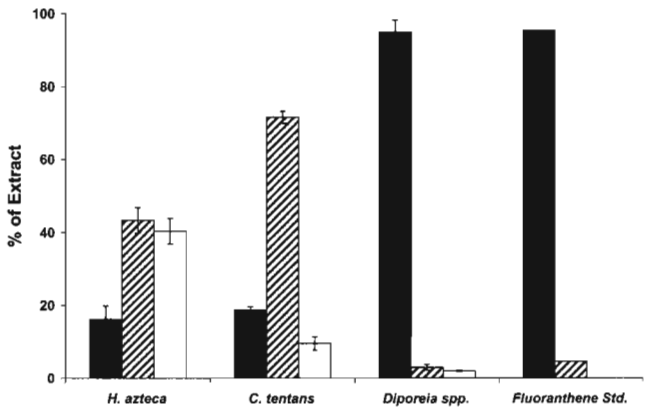

Fig. 2. Tissue composition of fluoranthene equivalents for *Hyalella azteca*, *Chironomus tentans*, and *Diporeia* following 48-h water-only exposure to fluoranthene. Parent compound; polar metabolites; aqueous metabolites. Black = parent; hatched = polar metabolites; white = aqueous metabolites.

netic parameters within species, the log BCF values for pentachlorobenzene, calculated from parameters k_u and k_e, were relatively constant across exposure concentrations (Table 1). Log BCF values ranged from 2.66 to 2.92 and 3.24 to 3.73 for *C. tentans* and *Diporeia*, respectively. The values for *H. azteca* were intermediate between the two, ranging from 3.01 to 3.14. These log BCF values are reflective of the relative difference in lipid content among the species. The lipid levels for *C. tentans*, *H. azteca*, and *Diporeia* were measured at the beginning of the experiments using a spectrophotometric technique [19] and were approximately 1.2 ± 0.5, 1.8 ± 0.5, and 5.0 ± 1.6% of wet weights, respectively.

Biotransformation

In a preliminary exposure, *C. tentans*, *H. azteca*, and *Diporeia* were each screened to quantify their ability to biotransform pentachlorobenzene. Following a 10-d exposure to pentachlorobenzene, no evidence was seen that any of the organisms were capable of biotransforming pentachlorobenzene (Fig. 1). Mass balance ranged from 77 to 116%; therefore, all radioactivity in the organisms was considered to be parent compound.

In the previous study by Schuler et al. [9] examining fluoranthene toxicity, biotransformation was quantified after 48 h of exposure for *C. tentans* and *H. azteca*. Based on the toxicokinetics of fluoranthene biotransformation from Nuutinen et al. [20], it was assumed that metabolite concentrations were at steady state. To verify this assumption for *C. tentans* and *H. azteca*, a separate exposure was performed specifically examining biotransformation over 10 d. Since in the first experiment *Diporeia* had a limited ability to biotransform organic chemicals, it was not necessary for them to be included in this second experiment.

Using the same techniques as described in Schuler et al. [9], the biotransformation of fluoranthene in *C. tentans* and *H. azteca* was assessed using a modification of a standard lipid extraction. This extraction produced organic, aqueous, and bound residue phases. The organic phase (chloroform) contained the parent compound and the organic-extractable polar metabolites, which included the biotransformation products of phase 1 reactions. The aqueous phase (methanol/water) contained the water-soluble metabolites (conjugated metabolites), and the residual tissue contained the bound (unextractable) fraction. Mass balance for this extract ranged from 76 to 91%. The results of this experiment for fluoranthene biotransformation indicated that the concentration of fluoranthene in the water seemed to have relatively little effect on the rate at which metabolites were formed in *H. azteca* and *C. tentans*. After 48 h of exposure, parent compound among all exposures averaged approximately 17% of the total ^{14}C activity in *H. azteca*. Of the remaining activity, approximately 43% was present as organic extractable polar metabolites and approximately 40% as aqueous metabolites, and negligible amounts were determined as bound residues (Fig. 2). The polar metabolites are assumed to be phase 1 metabolites, while the aqueous metabolites are assumed to be mostly phase 2 metabolites but may contain some very polar phase 1 compounds. In the *C. tentans* exposures, the amount of parent compound averaged approximately 19% in the organism after 48 h. Of the remaining radioactivity, approximately 70% was associated with polar metabolites and 10% as aqueous metabolites (Fig. 2). The bound fraction in the *C. tentans* also was considered negligible.

Mortality LR50

The design of these experiments allowed replicate beakers to be nondestructively sampled at each time point, thus allowing LR50 concentrations to be determined at multiple times during the time course of each exposure. Mortality was evaluated at 4 and 10 d for *C. tentans* and at 4, 10, and 28 d for *H. azteca* and *Diporeia*. However, because of limited mortality, LR50 estimates for *Diporeia* were calculated only at 28 d. An additional LR50 value was estimated by extrapolation from the mortality data at 20 d. As shown in Figure 3, the pentachlorobenzene LR50 concentrations were calculated from the mean internal tissue concentration from dead organisms in each exposure concentration at specified times. Generally, the plot of mean lethal body residue versus mortality generated the typical dose–response toxicity curve. In addition, LR50 values declined significantly with exposure time for each species. For example, LR50 values for *C. tentans* declined from 1.20 $\mu mol \cdot g^{-1}$ wet weight at 2 d to 0.81 $\mu mol \cdot g^{-1}$ wet weight at 10 d. The LR50 values for *H. azteca* declined from 1.51 $\mu mol \cdot g^{-1}$ wet weight at 4 d to 0.71 $\mu mol \cdot g^{-1}$ wet weight at 28 d. The LR50 values for *Diporeia* ranged from 5.0 $\mu mol \cdot g^{-1}$ wet weight at 20 d to 2.75 $\mu mol \cdot g^{-1}$ wet weight at 28 d (Table 2).

Fluoranthene lethal residue data expressed as total equiv-

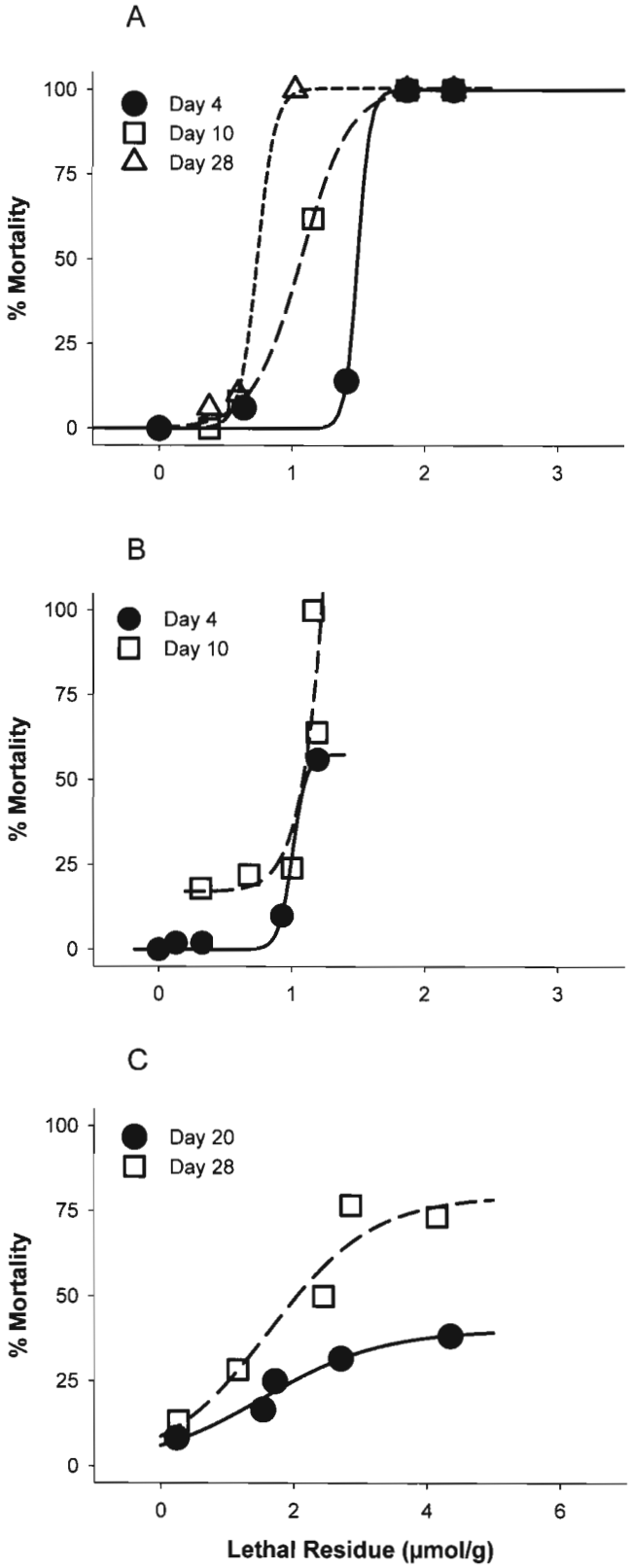

Fig. 3. Dose–response curves for (**A**) *Hyalella azteca*, (**B**) *Chironomus tentans*, and (**C**) *Diporeia* generated from pentachlorobenzene residues in dead organisms and percent mortality.

alents (parent compound and both phase 1 and phase 2 metabolites) from the earlier study were recalculated to reflect the toxic fraction (parent plus organically extractable metabolites) and are summarized in Table 3. The toxic fraction was determined from the metabolite data in the previous study since both the toxicity and the biotransformation data were collected simultaneously. Similar to results from the pentachlorobenzene studies, the LR50 concentrations, expressed as toxic fluoranthene equivalents, significantly declined with time for each species. The temporal response of LR50 concentrations of total fluoranthene equivalents in *C. tentans*, *H. azteca*, and *Diporeia* decreased two- to fourfold over the time course of the exposures.

The LR50 concentrations for pentachlorobenzene exhibited only a slight time dependence for *C. tentans*, *H. azteca*, and *Diporeia* compared to the fluoranthene exposures, with values dropping approximately twofold over the time course of the exposures. The data were modeled for both compounds using the DAM (Fig. 4). The time-dependent toxicity was determined using LR50 values together with a second measure of toxicity, the mean lethal residue (MLR50). The MLR50 is defined as the mean lethal residue of dead organisms in a given treatment level at the exposure duration corresponding to the lethal time at 50% mortality (LT50) [11]. The model input and output parameters are presented in Table 4 as well as the estimated $LR50_\infty$. As shown in Figure 4, the temporal patterns in toxicity were similar for *C. tentans* and *H. azteca*, where the lethal residue required to cause 50% mortality decreased fairly rapidly until approximately 120 h, after which the lethal residue approached incipient lethal residue. *Diporeia* LR50 values also dropped rapidly; however, no incipient value was obtained by the end of the 28-d exposure.

DISCUSSION

The overall goals of this research were to evaluate and compare the temporal component of pentachlorobenzene lethal body residues in three aquatic invertebrate species and determine the applicability of using lethal body residues as a dose metric for evaluating the toxicity among two different classes of nonpolar organic chemicals: a polycyclic aromatic hydrocarbon and a chlorinated benzene. To make appropriate interspecies comparisons, previous research has demonstrated the importance of understanding or identifying the factors that modify the toxicity of nonpolar chemicals, including lipid content, biotransformation, and toxicokinetic status.

To gain a better understanding of the time-dependent toxicity of pentachlorobenzene and fluoranthene, it was important to determine the toxicokinetic parameters over a range of exposure concentrations. For pentachlorobenzene, no trends were observed in the uptake and elimination rate coefficients with exposure concentration among the three test organisms. This is similar to the previous work using fluoranthene, where the rate coefficients were somewhat variable but the BCF values relatively constant. These findings also agree with those from a recent study by Landrum et al. [8] where the toxicokinetic parameters for *H. azteca* exposed to pentachlorobenzene were evaluated over a wide range of concentrations. Although the authors did report a decreasing trend in the toxicokinetic rate coefficients with increasing exposure concentration, the BCF data corresponding to the range of concentrations employed in the present study were fairly constant for concentrations above 0.7 $\mu mol \cdot L^{-1}$ ($\sim$175 $\mu g \cdot L^{-1}$). This suggests that exposure concentration has relatively little effect on the toxicokinetics up until the point of death. With the limited data available from the literature, it is unclear whether this relationship holds true for all narcotic compounds or for compounds acting via a specific mechanism of action.

Table 2. LR50[a] wet weight and LR50 lipid values (μmol·g^{-1}) for pentachlorobenzene in *Hyalella azteca*, *Chironomus tentans*, and *Diporeia* spp.

Organism	Time (d)	LR50 (μmol·g^{-1} wet wt)	95% CI[b]	LR50 (μmol·g^{-1} lipid)	95% CI
H. azteca	4	1.51	(1.40–1.63)	83.9	(77.9–90.0)
	10	0.95	(0.88–1.03)	52.8	(48.8–57.2)
	28	0.71	(0.66–0.77)	39.4	(36.4–42.4)
C. tentans	4	1.20	(0.96–1.44)	100.3	(80.4–120.3)
	10	0.81	(0.69–0.93)	67.4	(57.2–77.5)
Diporeia spp.	20	5.00[c]	—	100.0[c]	—
	28	2.75	(2.18–3.32)	55.0	(43.6–66.4)

[a] LR50 = the body residue responsible for 50% mortality.
[b] 95% CI = the 95% confidence interval.
[c] Estimate determined from extrapolating mortality data (see Fig. 3).

Pentachlorobenzene was selected for use in this study because it is not readily biotransformed by aquatic organisms. This was validated in this study for each of the test species. Although the test compound was relatively pure (>96%), some of the impurity was determined to be tetrachlorobenzene (Fig. 1). It is assumed that the presence of this impurity did not influence the outcome of the body residue data (data were determined as total equivalents from ^{14}C compounds) since tetrachlorobenzene, like pentachlorobenzene, is expected to act via narcosis. In addition, tetrachlorobenzene did not appear to be biotransformed, as no biotransformation products were detected following a 10-d exposure (Fig. 1).

In this study, several measures for determining the body residue causing 50% mortality were examined to determine their utility in predicting 50% mortality. The first method, the critical body residue (CBR), used BCF and LC50 estimates to determine the body residue associated with 50% mortality [1,2]. The BCF values used in the calculations of the CBR for fluoranthene were obtained from data in Schuler et al. [9]. These data were then recalculated on the basis of the determination of the toxic equivalent fraction (parent compound and phase 1 compounds). This approach assumes that the exposure concentration causes no systematic change in the toxicokinetics over the range of water concentrations employed (validated in this study). Second, the LR50 is the accumulated internal tissue concentration of a chemical at a specified time that yields 50% mortality. Finally, the MLR is the mean lethal residue corresponding to the LT50 at a specific exposure level, where the LT50 is the time at which a specific exposure is expected to cause 50% mortality. As seen in Figure 4, the lethal body residue data are similar, independent of which dose metric is used (CBR, LR50, or MLR50). However, to maintain some standardization in the literature, the actual terminology should not be used interchangeably.

Time dependence

A time-dependent relationship was observed between toxicity and body residue. As expected, the body residue causing 50% mortality for *H. azteca* and *C. tentans* decreased relatively quickly during short exposure times and then leveled out to an asymptotic value with longer exposures. This result for pentachlorobenzene is similar to the data previously collected for these two species using fluoranthene (Fig. 4). The reason for this decrease in lethal residues required to cause 50% mortality is due to the narcotic property of pentachlorobenzene. Narcosis occurs as a result of a chemical partitioning into a cell membrane, thereby disrupting its normal function, and, by definition, narcosis is expected to be reversible in that the exposed organism is capable of "fixing" the damage following removal of the chemical. However, for constant exposures beyond the time required to reach steady state, the continued accumulation of damage by the organism

Table 3. LR50[a] wet weight and LR50 lipid values (μmol·g^{-1}) as toxic fluoranthene equivalents

Organism	Time (d)	LR50 (μmol·g^{-1} wet wt)	95% CI[b]	LR50 (μmol·g^{-1} lipid)	95% CI
Hyalella azteca (exp. 1)	5	2.25	1.98–2.60	90.5	83.7–97.7
	10	1.14	1.00–1.30	55.0	47.8–63.5
	28	0.61	0.53–0.69	32.0	25.8–39.5
H. azteca (exp. 2)	5	1.48	1.31–1.58	74.0	59.6–91.9
	10	0.84	0.75–0.96	44.3	39.4–49.9
	28	0.56	0.50–0.64	31.9	28.1–36.2
Chironomus tentans (exp. 1)	2	0.38	0.34–0.44	30.0	26.8–34.8
	4	0.22	0.19–0.26	17.1	11.3–26.9
	10	0.15	0.13–0.17	12.3	7.8–17.8
C. tentans (exp. 2)	2	—	—	—	—
	4	0.23	0.18–0.28	16.9	11.2–26.8
	10	0.18	0.10–0.24	13.1	8.7–17.6
Diporeia spp.	10	9.97[c]	—	213.5	—
	28	3.67	2.32–5.80	85.3	65.5–111.3

[a] LR50 = the body residue responsible for 50% mortality.
[b] 95% CI = the 95% confidence interval.
[c] Estimate determined from extrapolating mortality data. Data recalculated using the fluoranthene toxic fraction determined from Schuler et al. [9].

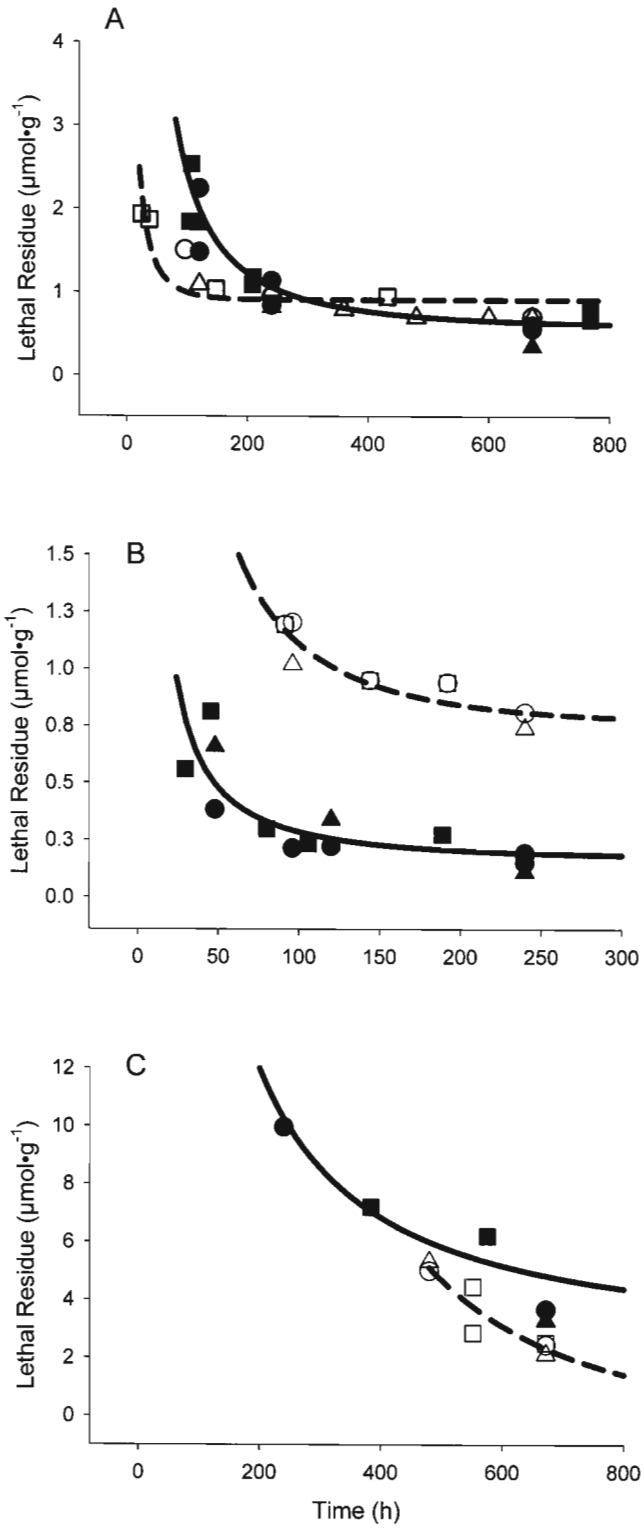

Fig. 4. Lethal body residues of fluoranthene toxic equivalents (solid line) and pentachlorobenzene (dashed line) producing 50% mortality over time for (**A**) *Hyalella azteca*, (**B**) *Chironomus tentans*, and (**C**) *Diporeia* spp. The closed symbols represent fluoranthene residues, and the open symbols depict pentachlorobenzene residues. ■ = LR50 (lethal residue for 50% mortality), ● = MLR (mean lethal residue for 50% mortality), ▲ = CBR (critical body residue for 50% mortality).

leads to increased mortality at reduced body residues. The temporal changes were modeled using the DAM, which estimates the time required for the damage to be repaired (k_r). The time dependence of the pentachlorobenzene body residues in the early portions of the curve were not as dramatic as compared to those for the fluoranthene residues. In addition to other factors, a potential impact exists of biotransformation through the early time course for fluoranthene that allows for reduction in the fluoranthene toxicity over time.

The results of the DAM for *H. azteca* exposed to pentachlorobenzene in this study were comparable to those of Landrum et al. [8]. The calculated $LR50_\infty$ values were within approximately a factor of two on a wet-weight basis for the two studies. For the *Diporeia* exposure, the model fit to the data was not significant over the time course of the experiment. Although we were not able to obtain good model estimates, a best-fit line was applied to the data to show the trend in the data.

Species and chemical comparisons

The acute toxicity of pentachlorobenzene to the three invertebrate species tested ranged from 1.5 to 5.0 μmol·g^{-1} wet weight. No significant differences were observed in LR50 wet-weight values over a 10-d exposure period between *H. azteca* and *C. tentans*. For *Diporeia*, mortality was limited until approximately 28 d. The 28-d LR50 wet-weight values for *Diporeia* were significantly higher than the other two test species. Generally, these lethal body residues were similar to the expected range of 2 to 8 μmol·g^{-1} wet weight for acute exposures to narcotic chemicals for fish [1,2,12] and within the range of those previously reported for aquatic invertebrates exposed to narcotic chemicals. For example, a recent study by Leslie et al. [14] determined the CBR of *Chironomus riparius* exposed to 1,2,3,4-tetrachlorobenzene to be 1.36 μmol·g^{-1} wet weight. In a separate study, these authors determined the 96-h CBR of *C. riparius* and *Lumbriculus variegatus* exposed to a mixture of chlorinated benzenes as 0.53 and >2.0 μmol·g^{-1} wet weight, respectively [21]. In addition, our range of lethal residues for pentachlorobenzene compare well to the 1.29 to 2.34 μmol·g^{-1} determined for the invertebrate *Eisenia andrei* [22]. Therefore, based on the lethal residue concentrations and the absence of biotransformation, it is expected that pentachlorobenzene causes toxicity via narcosis for the three invertebrates tested.

As indicated previously, some of the variation in the pentachlorobenzene lethal residues among species used in this study can be attributed to several factors, including lipid content. Normalizing whole-body lethal residues by the average lipid content of each species has been previously shown to reduce the range and variation of lethal residues among fish and invertebrates [23,24]. This occurs because the nonpolar chemicals are expected to strongly partition into the lipid compartments of the organism. Since these compounds preferentially partition into lipids, the total lipid content is a suitable surrogate for membrane lipids, which are the suggested target site for narcotic chemicals. Thus, the higher the lipid content of an organism, the greater the body residue required to achieve an effect using wet-weight tissue concentrations. This is seen in this study by comparing the pentachlorobenzene residues among the three species, where the organism with the highest lipid content (*Diporeia*) was more than twice as tolerant as *H. azteca* and *C. tentans*. Following lipid normalization, no significant difference was observed among the acute pentachlorobenzene LR50 concentrations for *C. tentans* or *H. azteca* (Table 2). The residues for *Diporeia* were somewhat lower but within the expected range for toxicity caused by narcosis. This result is comparable to the fluoranthene study, where lipid normalizing the lethal residue data reduced the variation of

Table 4. Input and output parameters for pentachlorobenzene and fluoranthene (toxic equivalents) in *Hyalella azteca, Chironomus tentans,* and *Diporeia* spp. from the damage assessment model. Values in parentheses represent ±1 standard deviation

Organism	k_e[a] (h^{-1})	D_L/k_a[b] ($\mu mol \cdot g^{-1} \cdot h$)	k_r[c] (h^{-1})	$LR50_\infty$[d] ($\mu mol \cdot g^{-1}$ wet wt)
H. azteca				
Pentachlorobenzene	0.030	20.5 (±2.9)	0.044 (±0.008)	0.92
Fluoranthene	0.028	146.4 (±13.9)	0.004 (±0.001)	0.59
C. tentans				
Pentachlorobenzene	0.293	63.1 (±7.4)	0.012 (±0.002)	0.76
Fluoranthene	0.022	17.8 (±3.1)	0.010 (±0.005)	0.18
Diporeia spp.				
Pentachlorobenzene	0.004	—	—	—
Fluoranthene	0.002	1,053 (±228)	0.003 (±0.001)	3.00

[a] k_e = total compound elimination rate constant (h^{-1}).
[b] D_L/k_a = the toxic damage level required to cause 50% mortality.
[c] k_r = the first-order rate constant for damage recovery.
[d] $LR50_\infty$ = the modeled incipient lethal residue.

fluoranthene residues among species by an order of magnitude [9]; however, normalizing for lipids did not account for all the differences among species.

A second issue affecting toxicity that should be considered when making comparisons among species or chemicals is the relationship between exposure duration and the time to reach steady state among species. When making comparisons at selected exposure times, different species may be at different relative positions on the toxicokinetic curve. This can be seen in Figure 5, where the body residues for fluoranthene in *C. tentans* and *H. azteca* are at steady state by 4 d; however, *Diporeia* does not approach steady-state conditions until approximately 28 d. Steady state for pentachlorobenzene was reached at similar times to those in the fluoranthene exposures. Therefore, in these experiments it would be incorrect to make comparisons among the species at a selected exposure time of 10 d because the toxicities of *C. tentans* and *H. azteca* are controlled by differing factors (toxicodynamics), while the toxicity of *Diporeia* are influenced by toxicokinetics (uptake and elimination). Therefore, to normalize the toxicity to the same kinetically defined time frame, comparisons of lethal body residues among species and chemicals should be made at 4 d for *C. tentans* and *H. azteca* and 28 d for *Diporeia*. The influence of normalizing LR50 concentrations to account for lipid content and identifying similar kinetically defined exposure periods can be seen in the first panel of Figure 6. For the fluoranthene experiments, no significant differences exist in LR50 values based on total fluoranthene equivalents between amphipod species; however, the lethal residue in *C. tentans* is significantly lower. For pentachlorobenzene, the LR50 concentrations for *Diporeia* are significantly lower than those for *C. tentans* or *H. azteca*, suggesting that *Diporeia* are more sensitive; however, the values are within the range expected for nonpolar compounds. The somewhat lower values may be partially due to the organism not quite reaching steady state at the end of the 28-d exposure. The estimated time to reach steady state calculated from the kinetic data was approximately 36 d.

Some of the variation in the lethal residues among species in the fluoranthene experiments may have been due to their different biotransformation capacities. When the lethal residues are compared among the three species, *C. tentans* residues were significantly lower compared to the two amphipod species following lipid normalization. The acute LR50 values for *C. tentans* were 0.20 and 0.25 $\mu mol \cdot g^{-1}$ wet weight, which is lower than values associated with narcotic response to nonpolar chemicals [1]. The difference was hypothesized to be the result of a change from narcosis to a more specific mode of toxic action due to the formation of a toxic metabolite(s). The formation of a toxic metabolite(s) in *C. tentans* is further supported by examining the toxic fraction of the compound within the organism's tissues. The toxic fraction of the compound can be obtained by quantifying the organically extractable fraction of fluoranthene within the organism's tissues. The organically extractable fraction corresponds to parent compound and phase 1 metabolites. Both parent compound and phase 1 metabolites are expected to contribute to the narcotic body residue [24]. Because of the analytical methodology used to separate fluoranthene compounds, the organically extractable or toxic fraction of fluoranthene may potentially contain a mixture of several fluoranthene metabolites (primarily phenols); however, the contribution of each individual congener to the overall toxicity remains unknown. The contribution of the toxic fraction to the total equivalents for each of the three invertebrate species is shown in Figure 5. This verifies the assumption that the fraction of parent compound is relatively constant with time and concentration. The percentage of parent and metabolite compound collected in this study was different compared to the data collected in the earlier study [9]; however, the toxic fraction was consistent. The LR50 values corrected for the toxic fraction for *C. tentans* ranged from 0.38 $\mu mol \cdot g^{-1}$ wet weight at 2 d to 0.15 $\mu mol \cdot g^{-1}$ wet weight at 10 d, and the corrected LR50 values for *H. azteca* ranged from 2.25 $\mu mol \cdot g^{-1}$ wet weight at 5 d to 0.56 $\mu mol \cdot g^{-1}$ wet weight at 28 d. After correcting for the toxic fraction, the lethal residues for *H. azteca* are still within the range expected for narcosis and did not account for the difference between the two species. Similarly, correcting for the toxic fraction did not account for

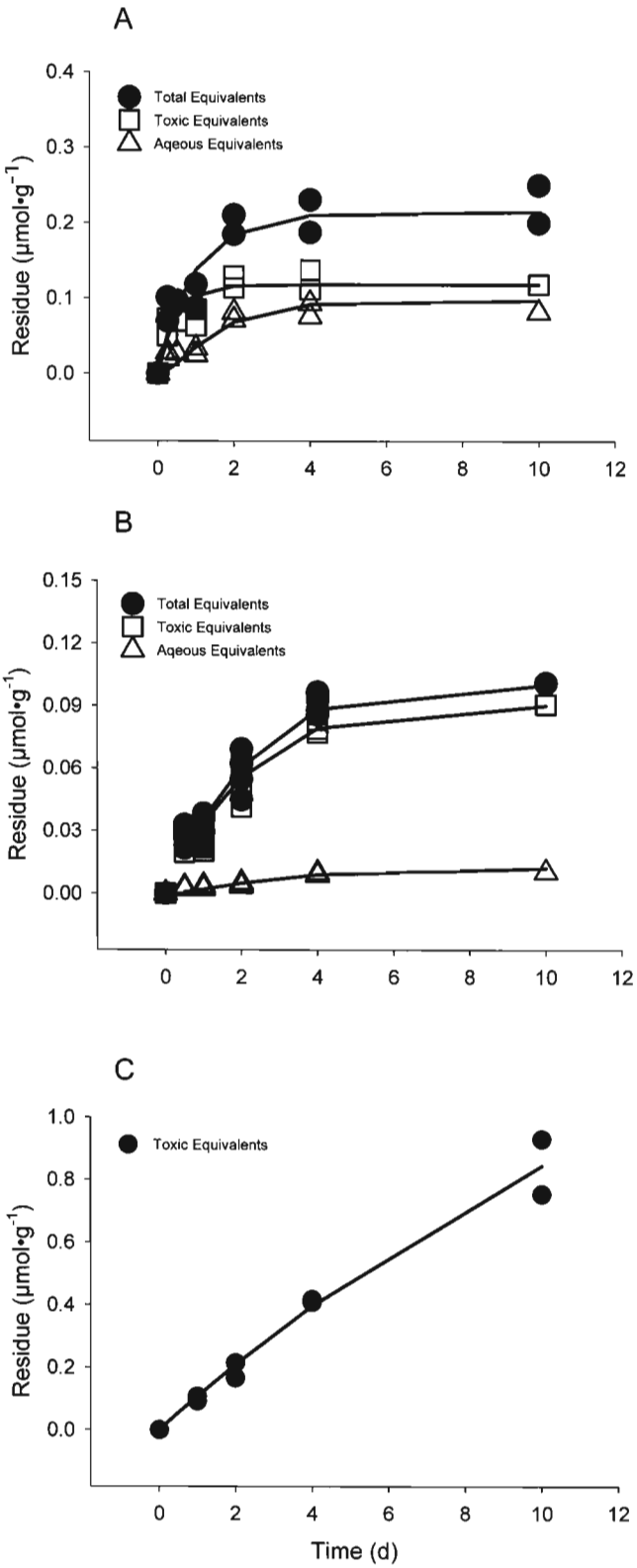

Fig. 5. Contribution of toxic fluoranthene equivalents to total fluoranthene equivalent lethal body residues producing 50% mortality over time for (**A**) *Hyalella azteca*, (**B**) *Chironomus tentans*, and (**C**) *Diporeia* spp.

the differences between *Diporeia* and *C. tentans*; however, it did bring the lethal residue data for two amphipods species more in line.

In addition to the potential formation of a toxic metabolite(s) in *C. tentans*, the difference in the toxicity of intermediate metabolites as a result of phase 1 metabolism may contribute significantly to the increased toxicity above baseline narcosis of *C. tentans*. Some of the likely reactive intermediates, such as epoxides, could shift lethal body residues into the range that would be expected for compounds acting via a specific mechanism of action. Epoxide formation was not directly quantified in this study because of analytical constraints; however, indirect evidence supports some potential involvement of epoxides in the overall toxicity. As previously demonstrated [9], some of the fluoranthene residues in the invertebrate extracts were determined to be bound in the tissues, which would suggest that some reactive intermediates were formed and became covalently bound to macromolecules within the organisms.

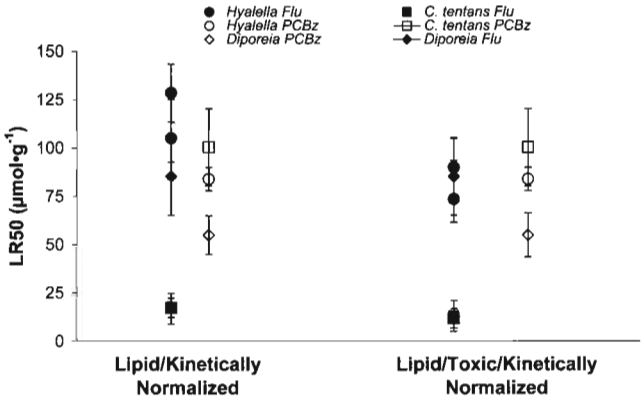

Fig. 6. Comparison of fluoranthene and pentachlorobenzene toxicity using $LR50_{(lipid)}$ (lethal residue for 50% mortality normalized to lipid) concentrations normalized for the toxic fraction for the three invertebrate species at their corresponding steady-state times (kinetically normalized). The toxic fraction is defined as parent compound plus organically extractable polar metabolites.

This further supports the possibility of a mode of action other than narcosis for *C. tentans*; however, an alternative explanation may be that *C. tentans* is simply more sensitive to nonpolar compounds than the two amphipod species. To further explore the hypothesis of a toxic metabolite formation in *C. tentans*, the lethal residues for the two chemicals were compared. Knowing that pentachlorobenzene is not biotransformed to any appreciable extent (Fig. 1) and assuming that pentachlorobenzene causes toxicity via narcosis [25], the LR50 data for fluoranthene and pentachlorobenzene were reexamined accounting for lipid content, toxicokinetic status, and biotransformation capacity for *H. azteca*, *C. tentans*, and *Diporeia* (Fig. 6, right panel). First, examining the fluoranthene data, normalizing for the toxic fraction reduced the variation among replicated exposures and species; however, the residue for *C. tentans* is still much lower. When the pentachlorobenzene data are considered, it is apparent that the sensitivity of *C. tentans* to fluoranthene is substantially greater than to the nonbiotransformed pentachlorobenzene. This further supports the hypothesis that the fluoranthene toxicity is driven by the formation of a toxic metabolite and that *C. tentans* is not specifically sensitive to narcotic acting contaminants.

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